

PROPERTIES OF COLLOIDS IN RELATION TO TISSUE STRUCTURE

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The study of fresh tissues under the microscope by dissection or compression methods or in hanging drops, is accompanied by several difficulties. The clear-cut pictures of the fixed and stained tissue are absent, as well as the fibrillar structures characteristic of fixed sections. The tissue colloid changes rapidly on removal from the body, swelling or coagulating. It becomes necessary, therefore, to ascertain what properties of colloids can be determined by optical or micro-physical methods, and what changes colloids of different kinds undergo when subjected to conditions under which fresh tissues are examined. Much of this work has already been done by the colloid chemists, and the experiments and conclusions of this paper are mostly repetitions of those experiments which are necessary to illustrate the essential points, together with such modifications as were found necessary in applying the subject to our special needs. The results are applicable both to the study of single cells and to masses of tissue.

The colloids used were gelatin, egg-albumin, fibrin, gluten, ascitic fluid, and others. Test tube and slide experiments were made, so that the results could be checked up in each case under the microscope.

RELATION OF CONSISTENCY, SIZE, AND REFRACTIVITY OF TISSUE COLLOIDS

The first series of experiments were carried out to test the relationship between consistency and refractivity. When fresh tissues remain under the microscope for some time, some of the elements, as the nuclei, and 'fibrillae,' appear much clearer. Their refractiveness increases. A similar series of changes can be studied in strips of water-soaked gelatin of equal size, when dehydrated with solutions of alcohol of varying strengths. A strip taken from a 40 per cent solution is smaller, tougher, and more refractive than the corresponding strip taken from a 30 per cent solution. The behavior may be summed up by saying that the smaller the size of a shrinking colloid, the greater the toughness (tensile strength) and refraction. This would indicate that the increase in refraction in nuclei and fibrils in freshly mounted tissues, removed from an available source of oxygen and in the presence of accumulated waste products, is due to a gelation or partial dehydration of

the colloids of these structures. The accompanying increase in toughness is well shown when the tissue is teased. The change in size is often measurable, as in the contraction of the clot in tissue cultures.

The 'stiffness' of a colloid solution further depends, to a considerable extent, on the distance between the walls of the container. The viscosity is such, that the narrower the vessel, the stiffer is the colloid. It is thus possible that a considerable proportion of the supporting properties of the intercellular connective tissue substance, which appears in the living as a structureless colloid, may be due to the position it holds with reference to the tissue cells bounding it.

RELATION OF CONCENTRATION TO OPTICAL DIFFERENTIATION

To what extent can morphological appearances, based on differences in states or concentrations of colloids, be differentiated under the microscope? Studies on gelatin, egg-albumen, globulins, and gluten solutions of varying dilutions, show that there are two modifying factors which must be considered in studying the minimal optical differences between various concentrations. These factors are the thickness of the layer and the state of the colloid, whether sol or gel. In appreciably thick layers one is able to make more delicate distinctions under the microscope, than in extremely thin layers, where the concentrations of a given colloid may vary considerably without optical change. Nevertheless, a very small amount of a colloid can change the optical properties of a medium. The gel state, for a given concentration, is optically distinguishable from the sol state. Differences of concentration in the gel state are more easily seen than corresponding differences of concentration in the sol. Changes from one state to the other are frequently met with in tissues. They are often aided or hastened for histological purposes by the use of reagents. It is probable that the freezing process in making frozen sections of tissues, leaves structures in the tissues which have been produced by a change of sol to gel state. Hardy ('99, p. 187) describes precipitation networks in frozen colloids. Such changes are reversible in gelatin, but irreversible in the case of some of the tissue constituents.

RELATION OF CONCENTRATION TO STRUCTURE ON FIXATION

A. Fisher ('99), Mann ('02), and Höber ('14), among others, have pointed out that a structureless colloid solution, such as egg-albumen or gelatin, exhibits, on fixation, a network. Hardy ('99) has applied this phenomenon to the interpretation of the structure of 'fixed' protoplasm and Isaacs ('16) has applied it to explain the appearance of connective tissue fibrillae as one of the post-mortem changes in preserved tissue. An additional factor, as Hardy ('99, p. 187) points out, is the apparent structural entity produced by 'shear strains' in colloids. These shear effects are doubly refractive, and correspond to some of the fibrillar

structures described in connective tissue and other cells. Collagen fibers can be simulated by exerting shear strains on the structureless jelly of freshly coagulated fibrin in clotting blood or plasma.

The pattern of a precipitated colloid is the same for concentrated and dilute solutions. The former, however, is denser and coarser, the latter more delicate and thin. This gives us an index to the state of the tissues at the time of their death, when one is studying fixed material. The more dilute regions leave, on precipitation, delicate networks, while the stiffer, more concentrated regions leave coarser and denser coagula. Such changes mark the transition from young to older tissue, and show the variations in water content with variations of physiological state.

FACTORS IN THE FORMATION OF THE DEHYDRATION AND PRECIPITATION PATTERNS

A. Fisher ('99) has described the variation in the size of precipitation granules when various colloid substances are treated with fixing agents. When the action of the fixative is complete, it is usually found that the granules have arranged themselves in the form of a network. The lines formed are often strikingly homogeneous and definite, and resemble fibers and fibrillae. A study of coagulation, drying, and precipitation in gelatin or egg-albumen shows the process of network formation to consist of two steps. First, there is a union of particles of the colloid substance to granules or crystals which may be visible under a magnification of 900 or even less. This is accompanied by the second process, which consists of a winding together of these granules, imbedded in the more liquid parts of the colloid, so that a network, resulting from the diffusion currents, is produced. This would seem to be compatible with Hardy's conception of colloidal solutions as consisting of colloidal particles floating in an extremely dilute solution of the colloid in the solvent (Mann, p. 47). The currents continue until all parts are precipitated. The precipitation process, as also the dehydration process, involves a separation or displacement of liquid from solid. With most fixatives, the liquid set free is enough to destroy their isotonicity, even for various parts of a cell, whether near or far from the surface. Others, as formalin, produce little change in the colloids, when used alone, and there is little change of size or separation of ingredients.

From this description, it is to be expected that each colloid will have its own precipitation pattern, since different substances form different sized precipitation granules (A. Fischer), and exhibit different degrees of viscosity. Likewise different fixatives produce different patterns because they precipitate granules of different sizes in a given colloid, and because the rate of action is different.

RELATION OF CLOUDY SWELLING TO FIXATION PATTERN

The action of acids on colloids has been treated by different authors, most recently and thoroughly by Martin H. Fischer ('15). Of special interest in this connection is the postmortem swelling of tissue following an increase in the acid content, and the precipitation, under the same conditions, of the colloids of the casein type, such as the globulins (cloudy swelling), and probably chromatin. The so-called zymogen and secretion granules may also fall in this class (Fischer). Fixation of such tissue is equivalent to precipitation of dilute colloids, and if the precipitated albuminous granules resist the solvent action of acids in the fixative, they become thoroughly wound up in the delicate precipitation network in the protoplasm of the cell.

EFFECT OF 'PHYSIOLOGICAL' SALT SOLUTIONS

A series of experiments were carried out to test the effects of 'physiological' salt solutions on the colloids of the cell. The action of salts in maintaining the outline of the cell may be explained on either an osmotic or a colloid basis. In either case it is evident that the different parts of the cell,—the nucleus and cytoplasm, are in equilibrium at any given moment as far as water content is concerned. As soon as the oxygen supply of the cell is decreased, we have an increase in the acid content, and the cell will swell if free water is present. A solution such as Ringer's or Locke's prevents this, the former by "the antagonistic action of salts and acids," the latter through this plus neutralization, since Locke's solution contains sodium bicarbonate. Any simple system which would prevent this increased absorption of water by the cytoplasm must do so also for the nucleus. The nucleus, however, is denser than the cytoplasm.

Experiments were performed to see if one solution could accomplish this. As the nucleus and cytoplasm may be composed of different colloids or of different densities of the same colloid, two systems were prepared. The first contained fibrin and gelatin in water equilibrium, the reaction being acid; the second, two strengths of gelatin under the same conditions. On treating either system with a salt solution, swelling could be reduced, but it soon became evident that unless the system was kept exactly in one state of hydration, the relative density, consistency, or refractiveness of one moment were not comparable to those of the next, because the different colloids, as well as the colloids of different strengths, absorb and lose water at different rates and the sizes do not change in proportion. A third possibility consists of the fact that the nucleus may be more, or less acid than the cytoplasm, under the same conditions, and therefore may absorb different amounts of water. From the fact that nuclei appear brighter in tissues undergoing the first postmortem changes, it seems possible that the nucleus may actually undergo some dehydration while the cytoplasm is becoming hydrated.

The application of this to living cells in Ringer's solution is evident. Acid is constantly being produced and must be neutralized or counteracted. As long as the water content of the nucleus and cytoplasm is kept exactly as in the original condition, we are justified in calling our observations on these cells normal. As soon as the conditions vary, the nucleus will become too tough or too liquid for the corresponding variation in the cytoplasm. This no doubt takes place in the body within certain limits, under varying physiological conditions, and accounts for the fact that cells can grow in tissue cultures, after being washed in salt solutions. Too great a variation, however, is fatal, as a result, no doubt, of solution of the cell constituents on the one hand or their coagulation on the other.

SUMMARY AND CONCLUSIONS

It is thus evident that when tissues are removed from the body for examination in the living condition, any change in refraction indicates a dehydration, a gelation or a solution process, which, beyond narrow limits, tends towards irreversible changes in the state of the cell colloids. Both dehydration and gelation increase the refraction. An optical change in the tissue colloids is noticeable only after considerable change in concentration, but is more readily noticeable if the change is from the sol to the gel state or the reverse. The pattern produced on coagulation or fixation is due to a shrinking and to diffusion currents, which cause the granules which are precipitated to whirl around, finally becoming wound up in the parts which coagulate more slowly. The pattern for a dilute and concentrated colloid is the same, the former yielding a more delicate fixation figure than the latter. The physiological state of a tissue at the time of fixation can thus be worked out. The action of physiological salt solutions in preventing swelling due to acid intoxication is not uniform for all the constituents of the cell. Slight variations in strength of the salt solution may give false impressions as to the relative densities of the cell constituents, because of the different rates of dehydration.

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A SIMPLE PARAFFIN RIBBON WINDER

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TWO FIGURES

Several years ago McClung designed a cylinder revolving on an axle borne on two standards for the purpose of picking up the paraffin ribbon as it left the knife of the microtome and winding it lightly around the drum. The value of such an apparatus is obvious. Long serial sections may be wound entire onto the cylinder doing away with the danger of scattering the ribbons by a sudden puff of wind as sometimes happens when they are laid on paper in the usual way. It further does away with the necessity of piecing portions of ribbons together on the slide since a continuous ribbon allows strips of exact length to be cut.

The writer designed a machine for the same purpose (described in the *Trans. Amer. Mic. Soc.*, December, 1913) to be used with the Leitz base sledge microtome which carries the knife much higher than most cutting machines. It is not nearly so simple to make, however, as the one to be described in this paper.

McClung's model has been adopted by one of the American microscope manufacturers. Since the device is so exceedingly useful and the price of the machine on the market is beyond the limits of the average student's resources, the writer recently made a ribbon winder that can be built in half an hour and costs approximately eleven cents. It is so simple that every student of histology or embryology can make one for his or her own use.

DESCRIPTION

The dimensions may vary with the materials at hand and the measurements of the machine pictured will be given merely to present a general idea of the size relations.

Base. 8 inches by $3\frac{1}{2}$ inches, $\frac{7}{8}$ inch thick.

Uprights. A one inch dowel stick was used although a broom handle or even a flat board would serve. A quarter inch hole was drilled through the center of the dowel which was then cut across the diameter of the hole. This gave two sticks with a semicircular groove in one end. Each of these sticks was now cut to an exact length (in this case $3\frac{7}{8}$ inches) and fitted into holes bored in the base the centers of which were $5\frac{3}{4}$ inches apart.

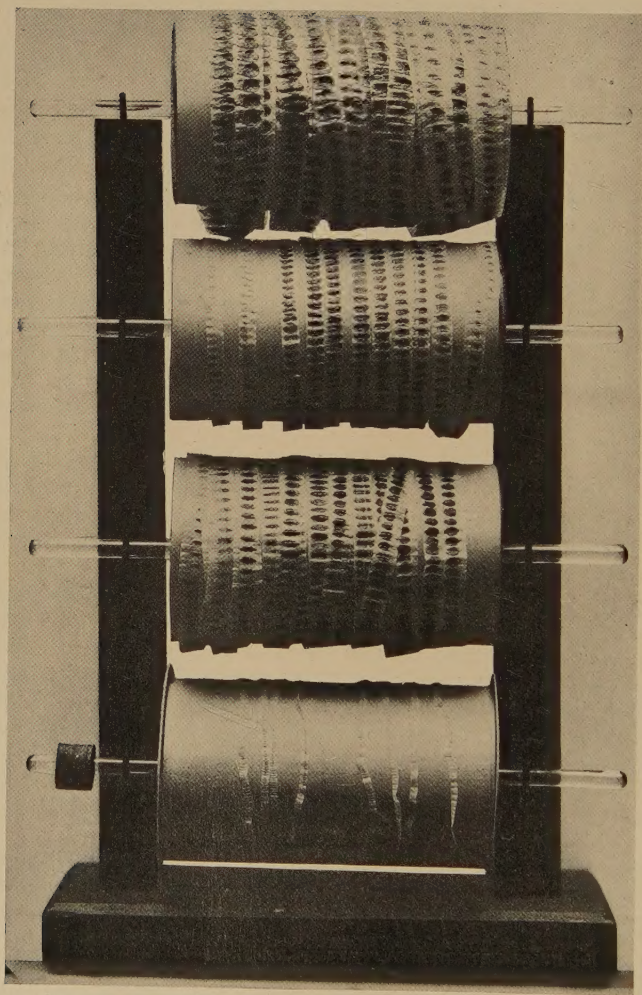


Figure 1

Cylinder. The cylinder was made of $2\frac{1}{2}$ inch cardboard mailing tube. I have made them of smaller sizes which work just as well. A twenty inch length of this tubing may be purchased for five cents and then cut into five lengths with a hand scroll saw or coping saw. Thin, flat corks, $2\frac{1}{2}$ inches in diameter, are bored to take a glass rod and are then fitted into the ends of the tube. A glass rod eight inches long is thrust through the holes. This forms the axle which fits into the semi-circular grooves in the uprights as shown in figure 1. The tube is finally wrapped with paper to give a smooth surface and the apparatus is finished.

Some workers prefer the roller to have flanges on either end. These may be easily made by gluing disks of heavy cardboard against the ends of the drum as shown on the lowest cylinder in figure 2. On the

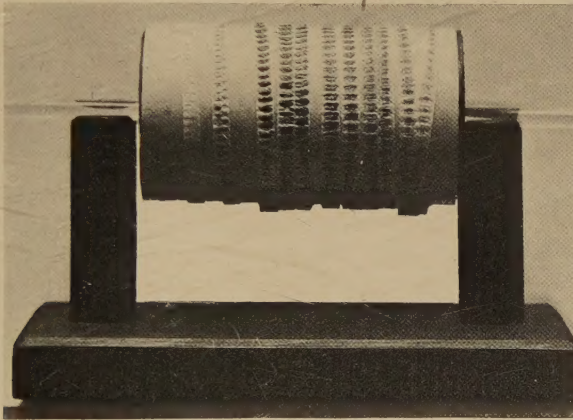


Figure 2

same drum is shown a cork fitted to the axle on one side which the worker can add should the glass rod not give sufficient purchase for easy turning.

Instead of a glass rod for the axle a quarter inch dowel might be used. Dr. McClung has suggested that a wooden mailing tube would serve for the cylinder and might even cost less than the cylinder with cork ends described above. Since these mailing tubes are turned up on a lathe the ends show the centering marks and make it easier to locate and drill the center for the axle than with the corks.

When in use it is necessary to slide the apparatus side ways so that the ribbon is wound spirally. That the mechanism may move smoothly three so-called domes of silence (such as are used on chairs) may be fastened to the bottom. These, however, are not absolutely essential.

The ribbon may be wound over and over itself without danger of sticking. The ribbon on the top cylinder of figure 2 is between 40 and 50 feet long and is unbroken. It may be allowed to remain on the

drum in perfect safety until the worker has time to mount it. Recently I have allowed a ribbon to remain on the cylinder uncovered and subjected to the usual variations in a room for four weeks and at the end of the time mounted the ribbon without trouble and without loss of a section.

A storage rack for the cylinders. When a worker has several blocks of material to cut it is of convenience to do them all at one time. For this purpose a number of drums are necessary and to take care of the drums a storage rack was made as shown in figure 2. The dimensions are the same as for the last base described with the exception of the uprights which are two've inches high. When one cylinder is filled it is placed on the hooks fastened into the upright as shown in the photograph and a fresh cylinder is placed on the working base.

SOME APPARATUS FOR THE MICROSCOPICAL LABORATORY

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FIFTEEN FIGURES¹

As new needs in the work of a laboratory constantly arise with advancing knowledge, modifications in old apparatus or entirely new forms are developed to meet those needs. Fortunately also, progress in knowledge is usually all along the line, hence the special needs in any given part are met and overcome by advances in other parts.

The devices here described have grown up to meet our special needs, and have been rendered possible, in large part, by recent advances in applied electricity, and by the production of new forms of glass.

In order to present the matter in the most compact and intelligible form, each piece of apparatus is shown drawn to scale.

1. Movable stand for microscopical slide trays, and for reagent boards (fig. 1). This stand is of the correct dimensions for holding 37 of the standard slide trays (fig. 2) or a less number of reagent boards, or a combination of the two.

It is constructed of four posts, a top and three fixed shelves. The three compartments thus produced are fitted with runs so spaced that two of the slide trays are admitted. Next the fixed shelves, three slide trays or a reagent board can be inserted. For ease in moving the stand, the corner posts are supplied with furniture slides or casters.

The sides are covered with fiber-board; the front is open as shown. If desired, a door may be added to enclose the front. If this is done, the door should be so hinged that it can swing around to one side and be entirely out of the way when inserting or removing the slide trays or reagent boards.

In our hands these stands have been found of special help for the following purposes:

a. For classes in histology, or especially in embryology, each group of students is supplied with a stand in which are the histologic specimens or the embryologic series needed for a given period or study.

b. For advanced students or investigators it has been found convenient to have one of these beside the work table to hold the series being worked on, or to contain needed reagents, or to hold series and ribbons of sections that are being prepared. As a tray of ribbons

¹ Engravings of all the figures were supplied by the authors.

should be covered by an empty tray for protection against draughts or mechanical injury, it is necessary to have the runs far enough apart to receive at least two trays.

c. Finally, in an investigation in which it is necessary to have at hand for consultation a large number of series, embryologic or histo-

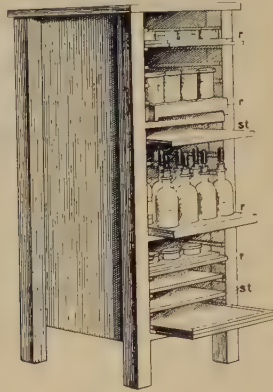


Fig. 1 Movable stand for slide trays and reagent boards. (About 1/19th natural size). *r*, reagent boards for glass bottles and jars; *st*, slide trays.



Fig. 2 Standard slide tray (about 1/7th natural size).

logic, the stand may be loaded with the needed series from the collection and placed beside the work table where they are convenient and also safe. In a word, it has the same advantages for specimens that a movable, revolving bookcase has for books.

2. Standard slide tray (fig. 2). The slide tray here shown is designed for 50 slides 25 x 75 mm., 10 in a row; 35 slides 38 x 75 mm., 7 slides in a row; 25 slides 50 x 75 mm., 5 in a row. Each row is separated by a low partition from the others. The partitions are only on one face. The smooth face is for ribbons of sections, etc. One side of the frame is grooved and the other tongued so that the trays may be

stacked, and remain in place. Besides the employment of the trays for general uses about the laboratory, they have been found most economical and convenient for storing the thousands of slides which go to make up a large embryologic and histologic collection. For the collection the slide trays are kept in lockers in which the runs allow the trays to be arranged in groups of five, more or less.

3. An electric paraffin melter (fig. 3). It is composed of a straight sided porcelain pitcher with a metal cover to exclude dust, placed in a galvanized iron receptacle lined with asbestos. A slot in the side for

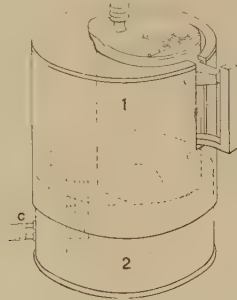


Fig. 3 Electric paraffin melter (about 1/10th natural size). 1, Porcelain pitcher with a metal top. This is the paraffin container and, as shown, its handle projects from a slit in the warming jacket. The pitcher rests on cross pieces. 2, Base supporting the pitcher and its jacket, and containing the electric sockets. In this one two lamps are shown. c, the electric supply cable. Both the metal cases are lined with asbestos paper.

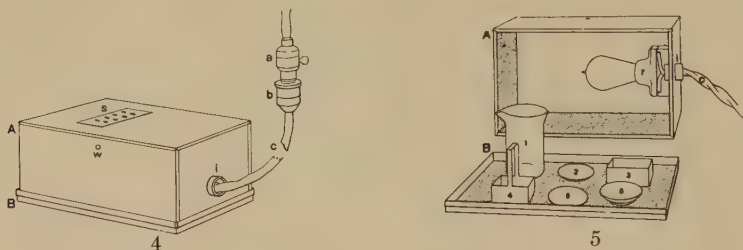
the handle allows that to project, and the pitcher of melted paraffin can be removed from the heater and returned without disturbing the heater.

The heater, as shown in the figure, is composed of two parts, the upper with cross pieces to hold the pitcher and the lower to contain the electric bulbs. We have found two 20 watt lamps sufficient. No regulator is used, for when the temperature is great enough to melt the paraffin the radiation is sufficient to hold the temperature between 55 and 60°C. We have found this a very convenient laboratory accessory.

4. Electric spreading plate and infiltrating oven (figs. 4 and 5). For spreading paraffin sections a level, smooth, warm surface on which to rest the glass slide is a great convenience, and it is almost necessary where the slide must remain for a considerable time on the warm plate without melting the paraffin, especially in glycogen staining. This plate is heated by an electric lamp of 10, 15, or 20 watts, depending on the season, and no regulation is necessary. If the lamp is at one end as shown in the figure, the plate is warmer at that end and one can get almost any desired temperature by moving the slide over the smooth surface nearer or farther from the lamp (see also fig. 12).

The metal box is lined by asbestos board, the spreading plate on top alone being uncovered. The box is placed in an asbestos lined, metal tray. In this tray may also be placed the infiltrating dishes, and a beaker for keeping paraffin melted.

This piece of apparatus is especially useful for the advanced student or investigator as no one else uses it and he can be confident that the tissues or embryos he is working with will not be disturbed or mixed up. It is perfectly safe too, if properly installed. That is, with a porcelain lamp receptacle and with proper insulation on the cable passing through the metal wall.



Figs. 4 and 5. Electric spreading plate and infiltrating oven (about 1/8th natural size). *A*, top, *B*, bottom; *a*, *b*, *c*, *i*, lampsocket, separable plug, cable and porcelain insulating tube; *r*, porcelain receptacle for the lamp; *w*, small window to show whether the lamp is burning; *1* and *2*, *5* and *6*, pyrex glass infiltrating dishes; *3* and *4*, metal infiltrating dishes.

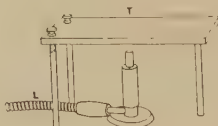
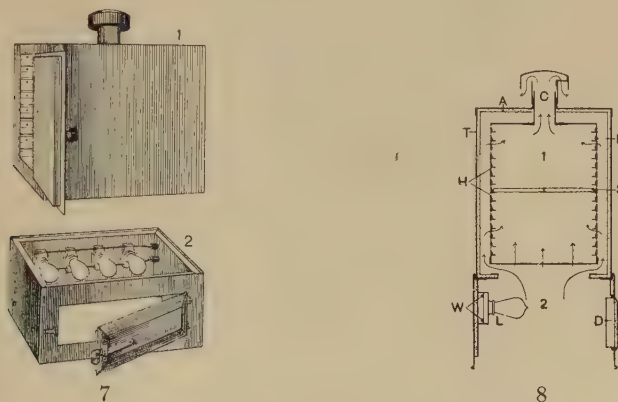


Fig. 6 Leveling plate for spreading paraffin sections, and for imbedding (about 1/10th natural size). *L*, small gas lamp; *T*, thick brass plate with three supporting legs, two with screws for leveling.

5. Gas-heated leveling table for spreading sections (fig. 6). If one must use a gas or an alcohol lamp for heating the spreading table it has been found by us that a heavy plate of brass with three legs is best, especially if the two at the end are fitted with a screw thread and a check nut to fix the legs in position when the table is level. The thick metal top can be made very smooth, and by heating one end any desired temperature can be obtained. The heavy metal top helps to make the table steady also. The ordinary thin metal tables cannot be made sufficiently level and the heat is not so uniform and continuous. Even if one has an electric spreader (figs. 4 and 5), such a table as this is very desirable for imbedding in paraffin, for the paraffin in the paper

boxes can be heated to any desired temperature before cooling the bottom and putting in the specimen.

6. Drying oven (figs. 7 and 8). For drying balsam mounted preparations, especially series of sections, and for drying spread sections on the slide, some form of steady heat, and a space free from dust is needed. The drying oven here shown was designed to receive 16 of the standard slide trays (fig. 2). It may be heated by gas or by the use of one or more electric bulbs.



Figs. 7 and 8 Warming and drying oven (fig. 7, 1/25th; fig. 8, 1/22d natural size).

Fig. 7 The oven proper (1), is 52 cm. long, 38.5 cm. wide and 45 cm. high, and has a capacity of 16 slide trays. The support (2), is 52 cm. long, 38.5 cm. wide and 25 cm. high. It is wired for four electric lamps.

Fig. 8 The oven (1) and support (2) in section. The arrows indicate the currents of warm air extending into and out of the oven. A, asbestos lining of the outer shell; B, one of the numerous ventilating holes from the air space into the oven; C, flue for the escape of the air at the top; H, runs for the slide trays (S); D, door of the support; W-L, wiring and lamp.

In general construction it is something like an incubator but is designed so that a current of warm air passes from the heating chamber below, up through the air jacket and through holes in the inner wall between each tray into the general space of the oven whence it circulates upward and escapes through a ventilating flue.

The outer wall is lined with asbestos to avoid radiation. For convenience it is composed of two parts, the lower serving as support and container for the heating device.

In the one here shown there are four lamp sockets. One can get almost any desired heat by using one or more of the lamps and by selecting lamps of greater wattage for the higher temperatures. By using a little care one can have almost any desired temperature without special regulation.

7. Devices for using artificial daylight in microscopic work (figs. 9, 10, 11, 12, 13, 14).² At last the great boon of an artificial daylight has been given to the worker with the microscope by means of the recently invented forms of glass light-filters. These have been prepared for the welsbach mantle light and for the incandescent electric light, especially the nitrogen-filled mazda lamp.

From our experience with these glass filters, the daylight glass should be ground on one or both sides with a very fine abrasive.



Fig. 9 Artificial daylight lantern for a single worker (about 1/15th natural size). *M*, microscope; *N*, nitrogen-filled mazda lamp of 100 watts; *a*, aperture for the daylight glass. This is about 5 cm. in diameter. The arched covering at the top is open on one side only, at the right in this figure. Compare figures 10 and 11, and for details of structure, figure 12. It will be noted that the table rail is cut out in front to avoid interference with the legs of the observer, and the table drawer is at the right and can be pulled out without moving. The seat is a revolving, adjustable piano stool.

² For a discussion of the requirements for the production of artificial daylight, and the means so far employed, and the uses of artificial daylight see:

Herbert E. Ives. Artificial Daylight. *Journal of the Franklin Institute*, vol. 177, May, 1914, pp. 471-499. 19 figures.

Simon H. Gage. Artificial Daylight for the Microscope. *Science*, N. S. vol. 42, October, 1915, pp. 534-536. One curve.

M. Luckiesh. Artificial Daylight. *Science*, N. S. vol. 42, November, 1915, pp. 764-765.

Some form of microscope lamp may be used, or if one is to use the nitrogen-filled mazda lamp, a cheap and efficient lantern can be cheaply constructed by any good tinner by using tin and making a square or round pail-like lantern. The lamp fixture is attached to the cover as shown in the diagrams, and opposite the filament of the lamp is an aperture in which is placed the daylight glass filter. As the lamp gives off much heat, the lantern must have large ventilating holes at the bottom and at the top.

On the inside the tin is coated with aluminum bronze. The reflecting surface increases the light passed through the glass filter and,

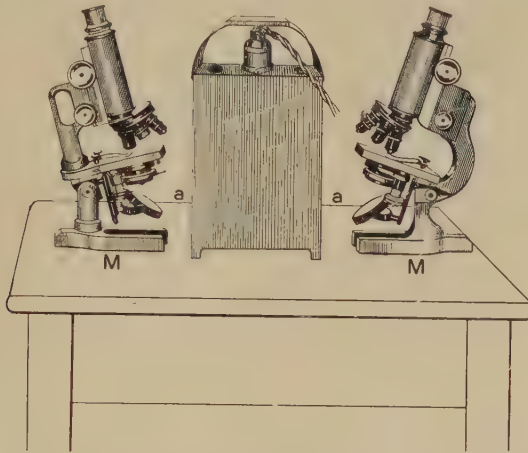


Fig. 10 Artificial daylight lantern for two workers on opposite sides of the table (about $1/9$ th natural size). *M*, microscopes on opposite sides of the table; *a*, opposite the apertures for the daylight glass (see figure 12 for the construction of the lantern). The arched covering over the lantern is open on only one side. Compare figure 11 in which the closed sides are toward the observer, while in this figure the open end faces the reader.

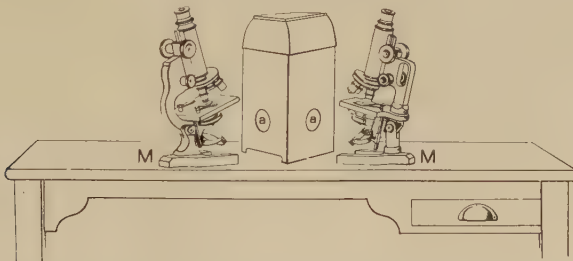


Fig. 11 Lantern for artificial daylight for two workers on the same side of the work table (about $1/15$ th natural size). *M*, microscopes placed obliquely to face the two openings for daylight glass in the lantern; *a*, daylight glass in adjacent sides of the lantern. (For the construction of the lantern see figure 12.)

more important still, it reflects the radiant energy instead of absorbing it as would a black surface. This aids in keeping the heat at a minimum. The outside of the lantern is painted dead-black to avoid unpleasant reflections. If but a single worker is to use the lantern, then only one aperture with the daylight glass is needed (fig. 9).

If two workers on opposite sides of the table are to use a single lantern, then there should be openings with the daylight glass on opposite sides of the lantern as shown in figures 10 and 12. If the two workers must be on the same side of the table or work-bench, then the two openings can be made as shown in figure 11, and the microscopes placed somewhat obliquely on the table.

Finally every laboratory has need of some means for demonstrating

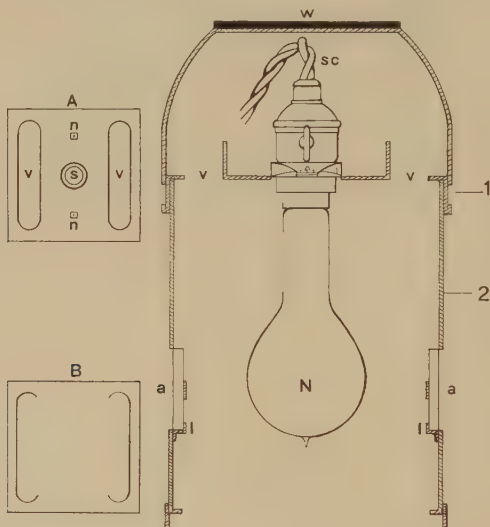
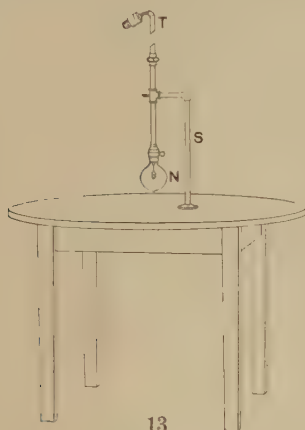


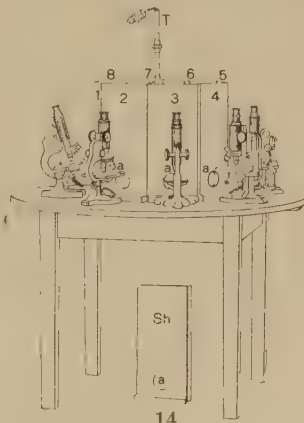
Fig. 12 Lantern for daylight glass in section (about 1/4th natura size). 1, top of the lantern supporting the lamp. It sets down on the lower part like the cover of a pail. *v*, ventilating slits. The tin from these slits is turned up at right angles; *sc*, the porcelain socket with key switch, and the asbestos insulated cable for the current; *N*, the 100 watt nitrogen-filled mazda lamp; *w*, the flat warming plate on the top of the cover. It is a brass plate about three millimeters thick. The temperature on this plate is about 40 to 45° C. and serves for spreading paraffin sections, etc. 2, the lower part of the lantern containing the daylight glass. It is square in cross section as shown by *A*, *B*.

a, daylight glass in the apertures of the lantern. The pieces of glass are about 5 cm. in diameter. *l*, lugs to hold the daylight glass in position; *A*, the partition containing the lamp socket seen from below; *v*, ventilating slits; *n*, nuts at the ends of the bolts holding the metal clamp for the lamp socket; *B*, bottom of the lantern. It is about 15 mm. from the table, thus permitting an intake space for air. Paraffin infiltration can be done here if one is careful. (*A*, *B*, are only about 1/9th natural size.)

the most difficult preparations or those showing a special feature in the most perfect manner. To meet this need a round table was constructed and a single lamp installed in the middle (fig. 13). The supply cable is received in the vertical tube about 2 meters above the floor to keep it entirely out of the way when it must be carried for some distance.



13



14

Figs. 13 and 14 Demonstration table for artificial daylight (about 1/25th natural size).

Fig. 13 shows the table with lamp and its fixture in position. *S*, standard screwed into a flange in the table; *N*, the nitrogen-filled lamp of 200 or 250 watts; *T*, tube for carrying the supply wires to the lamp. The tube passes through a collar at the end of the standard and may be fixed in any position by means of a set screw. The tube is about 150 cm. long and serves to hold the supply wires about 2 meters above the floor. The standard can be unscrewed if it is necessary to take the table through a narrow door.

Fig. 14 shows the table with the 8 shields containing daylight glass in the apertures (*a*), and also some microscopes in position. Below the table is one of the shields (*sh*) with the projecting wing at the right to lap over the next shield and prevent the escape of stray light.

In the demonstration table here shown there is room for 8 microscopes. Shields of wood or metal are set up and overlap at the edges to avoid stray light. Each shield contains a disc of the daylight glass, and all are lighted by the single nitrogen-filled mazda lamp of 200 or 250 watts in the center of the table (fig. 13). To avoid the very brilliant light extending upward, a disc of cardboard or tin is put over the lamp, but plenty of space must be left around the edges for ventilation. Some holes through the table top under the lamp should be made for ventilation also. In all of the devices shown the light is amply sufficient for all powers up to and including the 1.5 mm. oil immersion.

As pointed out by Brewster nearly a century ago, and still later by Nelson and Wright, the clearest microscopic images are obtained in the microscope when the source of light is relatively small. As these small

pieces of daylight glass are practically the sources of light, this prime requisite for a good light is furnished. It will be found in using the daylight glass, as here indicated, that one can utilize a considerably larger part of the aperture of the objective without the fogging and loss

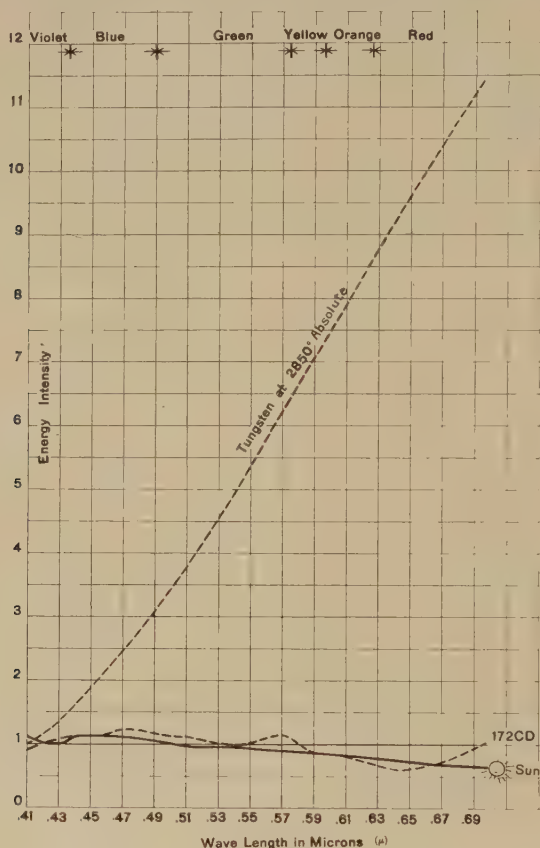


Fig. 15 Curve of sunlight, the light from a nitrogen-filled mazda lamp, and the mazda light filtered through daylight glass (172CD).

The numerals on the bottom show the wave lengths, and those on the left the intensity in the different parts of the visible spectrum.

of detail coming with a large aperture when one uses a large source of light like a window.

In figure 15 is shown a curve of the intensity in the different parts of the visible spectrum for sunlight, for the light of a nitrogen-filled mazda lamp, and for the lamp-light filtered through the daylight glass.

The great excess of red, orange, etc., in the unfiltered mazda lamp-light shown in this curve is most strikingly illustrated by looking at such a lamp burning out of doors in full daylight.

THE ANATOMY OF A THREE-LEGGED KITTEN

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From the Sheffield Scientific School and the Osborn Zoological Laboratory, Yale University

THREE FIGURES

The animal which is the source of this paper was an ordinary, tiger-striped, female kitten which came into the possession of the laboratory through the kindness of our colleague, Dr. Henry Laurens. It lived in the laboratory for a number of weeks, and when about six months old was killed and dissected to determine the exact nature of the anomaly.

The female parent of this kitten had previously given birth to other litters of normal offspring, and this animal which we studied was one of a litter of three, of which the other two were normal. Furthermore, all the members of a litter born since our specimen are normal. The male parent of our kitten is unknown.

The kitten, aside from its having only three legs, was a lively, healthy animal, with sleek fur, and a playful disposition. It balanced itself well, planting the right fore foot under the mid-line of its body to compensate for the missing left limb (fig. 1). There was no external evidence of any part of the left fore limb except a lump at the shoulder which indicated the presence of at least a part of the shoulder girdle, and the skin was drawn smoothly over the shoulder without any sign of puncture or folding.

As soon as the animal was chloroformed the skin was reflected from the left side of the body, and at once there came to view, beneath a thin layer of fascia, a point of bone (fig. 2). The shoulder muscles (deltoids, clavo-brachial, supra- and infra-spinatus, teres major and minor, and subscapular) were all



Fig. 1 Photograph of the three-legged kitten when about six months old. The right fore limb is swung under the body to compensate for the missing left limb.

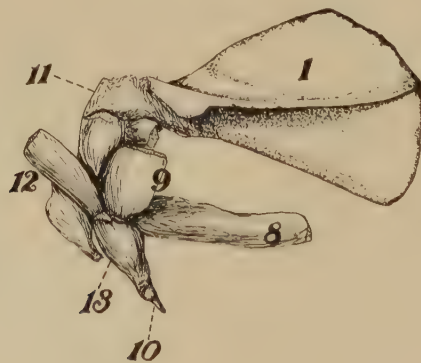


Fig. 2 Left scapula, rudimentary humerus, and deep shoulder muscles of the three-legged kitten. 1, scapula; 8, spinodeltoid; 9, acromiodeltoid; 10, distal end of the humerus; 11, capsule of the shoulder joint; 12, mass of muscle fibers representing the combined latissimus dorsi and the pectorals; 13, anomalous band of muscle fibers encircling the rudimentary shaft of the humerus. The unnamed stumps of muscles appearing below the joint capsule are those of the supra- and infraspinatus.

normal on the left side of the body with respect to origin, insertion, and innervation. This is not surprising, since the skeletal parts with which they are connected were only slightly abnormal, as described in detail later on.

The appearance of the left fore limb after the removal of most of the shoulder muscles is shown in figure 2. Two muscle anomalies were clearly evident. First, the fibers of the latissimus dorsi, pectoralis major and pectoralis minor muscles, all three of which were thinner than usual, but possessed of normal origins, became fused into a common sheet which was inserted on the inner surface of the humerus, where normally the latissimus dorsi alone is inserted, the normal place of insertion of the pectorals having failed to develop in this humerus. The second muscle anomaly was a mass of fibers which was wrapped completely around the humerus from the point of insertion of the pectorals and deltoids to within a short distance of the tip. Into this mass of muscle ran the musculospiral, musculocutaneous, median and ulnar nerves, but we feel no justification in offering a theory as to the homology of this anomalous mass of fibers. In addition to the above mentioned anomalies should be noted the entire absence of any recognizable triceps, biceps or coracobrachial muscles, a fact to be correlated with the absence of their points of origin from the abnormal left scapula and humerus. Possibly these three muscles are among those represented by the unnamed mass mentioned above.

The right and left scapular and humeral bones of the three-legged kitten are shown, drawn to the same scale, in figure 3. The clavicles, which are not represented, were normal on both sides. The left scapula differs from the right in the following respects: (a) the anterior superior border, owing to the narrow neck, forms a greater angle with the axillary border, (b) the spine of the left scapula, perhaps owing to the undeveloped state of the infraspinatus muscle, is lower than that of the right, (c) the suprascapular notch, on the anterior superior border, is missing, owing to the absence of the coracoid process, (d) the axillary border of the left scapula shows, in correlation with the abnormally narrow neck and head, a decided thickening at its

junction with the neck, (e) the neck of the left scapula is only about half the width of that of the right, (f) there is an entire absence from the left scapula of the coracoid process, including the areas for the attachment of the coracobrachialis and biceps muscles and the supraglenoid fossa, (g) correlated with the complete absence of the coracoid process, the glenoid cavity of the left scapula is imperfectly developed, possessing not more than half its usual area, and it is convex rather than concave. In connection with the above mentioned anomalies it should be noted that according to Jayne ('98) the coracoid process and the



Fig. 3 Outer aspect of the right and left scapular and humeral bones of the three-legged kitten, all drawn to the same scale. 1, left scapula; 2, left humerus; 3, epiphysial groove; 4, shaft; 5, right humerus; 6, abnormal depression in neck of left scapula; 7, right scapula; 8, epiphysial groove; 9, shaft.

upper portion of the glenoid cavity of the scapula develop in the cat from distinct ossification centers. Also, the work of Braus ('08) and of Harrison ('15) has shown that in Amphibia the anlagen of the shoulder girdle and of the fore limb are entirely distinct, since experimental removal of the latter does not prevent the development of the former.

The left humerus of this specimen is chiefly characterized by its representing so small a part of the normal bone. Like the right humerus the left shows a clearly defined epiphysial groove, but on the left humerus there is an entire absence of the lesser tuberosity, together with about half of the articular surface

adjoining that tuberosity. The part of the shaft present on the left humerus corresponds to the lateral surface of the normal bone from the anterior mid-line laterally to the deltoid ridge. It is everywhere covered with a thick layer of compact bone, and possesses no marrow cavity, its whole interior being filled with spongy bone.

What caused the production of this three-legged animal must probably await conclusive settlement until such anomalies are experimentally controlled, but taking into account all of the available evidence it would appear that after starting to grow out normally from the body the left fore limb bud encountered some obstacle which pressed it against the body, as well as checked its increase in length; such of it as had already grown then underwent further development into bone, muscle, connective tissue, etc.

MARCH 1916

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ON THE DEVELOPMENT OF THE BILIARY SYSTEM IN ANIMALS LACKING A GALL-BLADDER IN POST- NATAL LIFE

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TEN FIGURES

It is well known that the gall-bladder is always absent in some species of vertebrates. There seems to be no general rule governing the distribution of this peculiarity. Thus in the cyclostomes a gall-bladder is found in the myxinoids but is absent in the fully developed Petromyzon. It is present as a rule in fishes although absent in a few species of sharks. A gall-bladder is said to be present always in amphibians and reptiles; it is wanting in a number of birds including the pigeons. Among mammals no gall-bladder is found in a number of rodents and in many ruminants, pachyderms and some other ungulates. It is absent in the Cetacea. In other forms the gall-bladder may or may not be present. This is the case in several species of birds and in a few mammals. With the latter may be included man where congenital absence of the gall-bladder is an unusual anomaly. In man the gall-bladder alone may be lacking, or more rarely both the gall-bladder and the larger biliary ducts.²

Only three of the forms consistently lacking a gall-bladder are commonly available for embryological study. These are the

¹ This work was done with the aid of a grant from the Research Fund of the University of Minnesota.

² The comparative list given above is by no means a complete one. A much more detailed statement is to be found in the Milne Edwards, *Leçons sur la physiologie et l'anatomie comparée*, T. 6, p. 454, et seq. Later papers upon the partial or complete absence of the biliary system in man are: Hochstetter, *Arch. f. Anat.*, 1886; Latham, *Journ. of Anat.*, 1898; Bubenhofer, *Anat. Hefte*, 1905; and Beneke, *Marburger Universitätsprogramm*, 1905.

lamprey (*Petromyzon*), the pigeon and the rat. The development of the biliary system in these species will be taken up in the order given above.

PETROMYZON

I have made no study of *Petromyzon* material but include here a brief summary of the findings reported in the literature.

The early history of the liver in *Petromyzon* has been studied by Götte ('90), v. Kupffer ('93), Brachet ('97) and others. A complete biliary system is developed including a large gall-bladder and a common bile-duct of considerable diameter. These structures are lined with a high columnar epithelium which later becomes somewhat flattened and they are surrounded by definite mesenchymal sheaths. The biliary apparatus takes part in the general rotation of the gut which occurs in this form and it persists during the *Ammocoetes* or larval period.

The later history of the common bile-duct and gall-bladder has been followed out in some detail by Nestler ('90). He found that in an *Ammocoetes* 13 cm. in length the bile-duct still opened into the anterior end of the mid-gut and was connected distally with the gall-bladder. Near its posterior or intestinal end there was a short segment in which the lumen was almost completely obliterated by the increased height of the lining epithelium. In a specimen a centimeter longer the common bile-duct was still connected with the gall-bladder but its connection with the intestine was lost and its caudal end obliterated. In this region was found a collection of 'Zellballen' or follicles which were connected with the part of the duct still intact. In still later stages the duct proper was found to have entirely disappeared but the follicles persisted. Nestler apparently regarded these follicles as cystic remains of the common bile-ducts or of out-pouchings from it. The gall-bladder first became much reduced in size and the epithelium lining it increased in height. The surrounding sheath of mesenchymal tissue also was thickened. Later the structure was completely obliterated.

These observations were confirmed in the main by Bujor ('91) who, however, accepted the earlier view of Langerhans ('73)

that the follicles observed near the cranial end of the common bile-duct were pancreatic. This view has been confirmed by the recent careful researches of Picqué ('13).³

PIGEON

In the pigeon, as in birds generally, there are two ducts leading from the liver to the intestine. Of these the anterior or cranial one is commonly termed the ductus hepato-entericus. The posterior one is divided into two parts by the point of attachment of the gall-bladder. The portion between the hepatic trabeculae and minor ducts and the gall-bladder is called the ductus hepato-cysticus, and the part between the gall-bladder and intestine the ductus cysticus or ductus cystico-entericus. Since the pigeon, as a rule at least, loses its gall-bladder in the course of development, the terminology above is not always an appropriate one. For simplicity the two main ducts will be designated in the following description as the anterior and posterior hepatic ducts.

Brouha ('98) has published the only information which we have concerning the later development of the liver in the pigeon. He modelled and described two stages, embryos of 59 and 100 hours incubation respectively, in connection with his extensive study of the development of the liver in the chick. The liver forms from the floor of the foregut and the anterior intestinal portal, but the anlage, which is hollow in the chick, is solid at first in the pigeon. In Brouha's younger specimen the anlage was already partially divided into anterior and posterior diverticula as in other birds. In the older specimen this division was quite distinct and the anlage of the gall-bladder was represented by a distinct expansion of the left side of the extreme posterior end of the hepatic gutter at the point of origin of the posterior hepatic diverticulum or duct (Brouha, '98, pl. VIII, fig. II, V. B.). Brouha did not follow its later history.

³ Although Nestler does not describe a pancreas in *Petromyzon*, he figures and labels as pancreas a group of tubules in the wall of the mid-gut on the side opposite to the follicles associated with the ductus choledochus.

The youngest pigeon embryo I have examined is 7 mm. in length, of 72 hours incubation (Minn. E. C. 588). In development it stands between the two stages described by Brouha. The anterior and posterior divisions of the hepatic diverticulum are differentiated into short wide ducts which connect with a considerable network of hepatic trabeculae surrounding, in part, the large right omphalo-mesenteric vein. All three pancreatic anlagen are present. The dorsal one forms a pouch which is

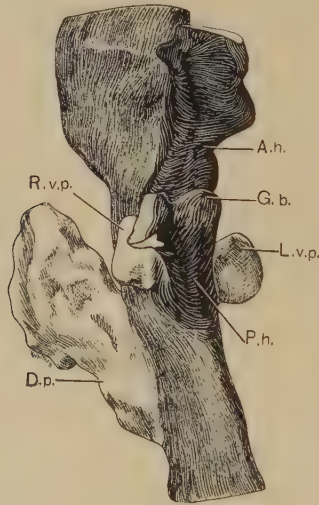


Fig. 1 A reconstruction of a portion of the mid-gut, liver and pancreas of a pigeon embryo 7 mm. long (Minn. E. C. 588). Right lateral view, $\times 50$. *A.h.*, anterior hepatic duct; *D.p.*, dorsal pancreas; *G.b.*, gall-bladder; *L.v.p.*, left ventral pancreas; *P.h.*, posterior hepatic duct; *R.v.p.*, right ventral pancreas.

almost as large in cross section as is the gut at this level. The ventral diverticula are smaller pouches which arise from the gut just above the posterior end of the hepatic gutter. A right lateral view of a reconstruction of this region is shown in figure 1. In the reconstruction the hepatic trabeculae are cut away near their origin from the hepatic diverticula. The posterior hepatic diverticulum is very short and ends by dividing into two parts. The right division becomes continuous with the hepatic trabeculae and represents the ductus hepato-cysticus of other

birds. The left division is the anlage of the gall-bladder and at this time is simply a short rounded swelling. This swelling is due almost entirely to the thickening of the epithelium, there being no true lumen in the cystic anlage (fig. 3).

The posterior hepatic diverticulum lengthens rapidly, extending forward and to the right. The cystic anlage is carried along

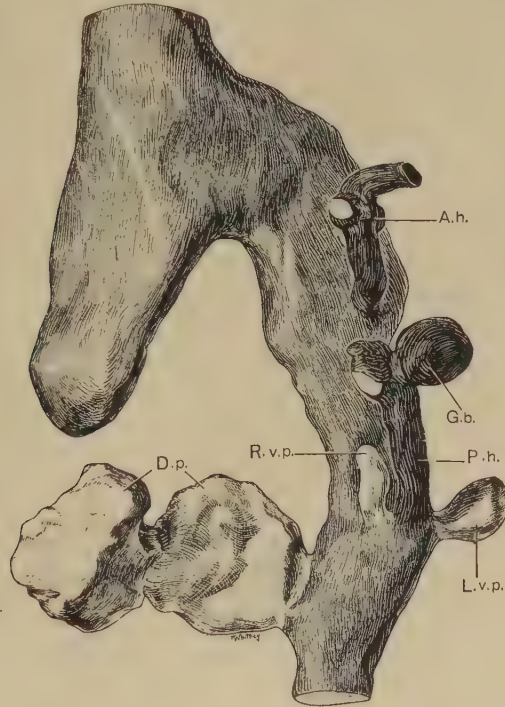


Fig. 2 A reconstruction of a portion of the mid-gut, liver and pancreas of a pigeon embryo 8 mm. long (Minn. E. C. 590). Right lateral view $\times 50$. Abbreviations as for figure 1.

with it and is soon located some distance from the gut in the margin of the hepatic parenchyma. The right branch of the bifurcation which connects with the hepatic trabeculae straightens out so that its axis falls in line with that of the main duct and the two form a single tube. The thickening representing the gall-bladder is strongly evaginated forming a small pouch which

joins with the posterior hepatic duct by a short broad neck. Its walls become thinner and its lumen is thereby widened, but as a whole it increases very little in size. An embryo (Minn. E. C. 590), 8 mm. in length and distinctly older than the one first described, shows these changes in progress. Figure 2 is a

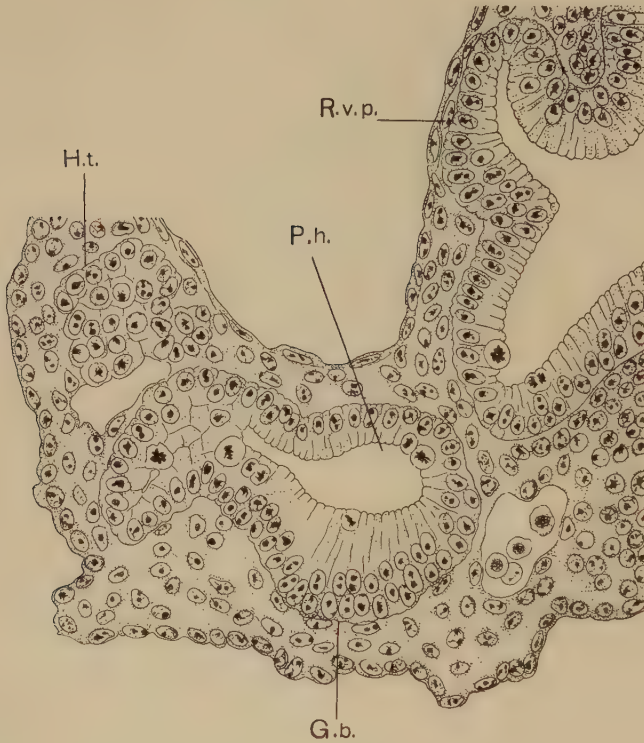


Fig. 3 Transverse section of the liver and the duodenal region of a pigeon embryo 7 mm. long (Minn. E. C. 588). $\times 400$. *G.b.*, gall-bladder; *H.t.*, hepatic trabeculae; *P.h.*, posterior hepatic duct; *R.v.p.*, right ventral pancreas.

right lateral view of a model of this specimen, and figure 4 a section through the long axis of the gall-bladder.

In later stages the entire mass of the liver is much enlarged. Both the anterior and posterior hepatic ducts increase rapidly in length and in caliber, but the anterior one grows more rapidly

than the posterior. Although the liver and its ducts continue to increase in size, the gall-bladder remains about constant until the embryo reaches a length of 12 to 15 mm. Thereafter the gall-bladder becomes somewhat smaller both absolutely and relatively than in earlier stages. Both the lumen and the walls of the sac tend to diminish, and folds or sacculations may appear in its fundic end. The surrounding mesenchyma begins to take the form of a definite sheath with concentrically placed



Fig. 4 Section through the long axis of the gall-bladder of a pigeon embryo 8 mm. long (Minn. E. C. 590). $\times 400$. *C.d.*, cystic duct; *G.b.*, gall-bladder.

nuclei. The neck or true cystic duct becomes elongated and slender. Figure 5, an oblique section of the gall-bladder of an embryo 15.5 mm. long (Minn. E. C. 586), shows these changes in progress.

With the growth of the liver the gall-bladder changes its position, being carried away from the margin of the liver parenchyma where it was formerly located. In later stages it comes to lie more and more in the posterior part of the dorsal ligament.

The cystic duct is reduced in caliber and the lumen becomes very small or is occluded. From the specimens which I have examined it appears that the bladder generally becomes separated from the duct and remains for a period as an isolated, thick-

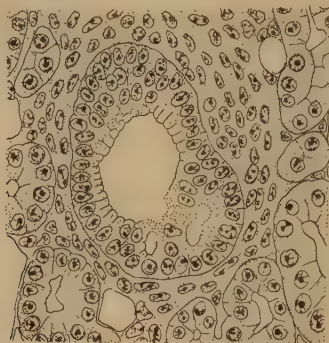


Fig. 5 Section through the long axis of the gall-bladder of a pigeon embryo 15.5 mm. long (Minn. E. C. 586). $\times 400$.

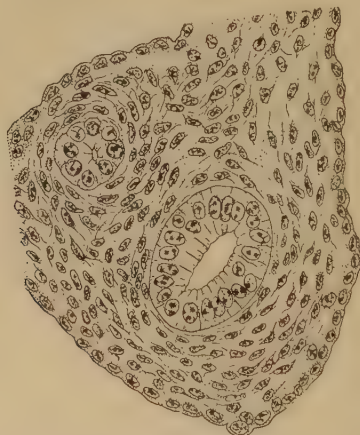


Fig. 6 Section through the gall-bladder and posterior hepatic duct of a pigeon embryo 26.8 mm. long (Minn. E. C. 596). $\times 400$. The gall-bladder is the larger of the two epithelial tubes.

walled cyst lying in the dorsal hepatic mesentery close to the posterior hepatic duct. This condition is shown in figure 6 of a section of the gall-bladder in an embryo 26.8 mm. long (Minn. E. C. 596).

Later the gall-bladder degenerates in most cases. In an embryo 45 mm.⁴ in length there was no definite cyst but in the region formerly occupied by the gall-bladder there was found a small cluster of deeply staining and degenerating cells which probably represented its remains. There was no trace of this

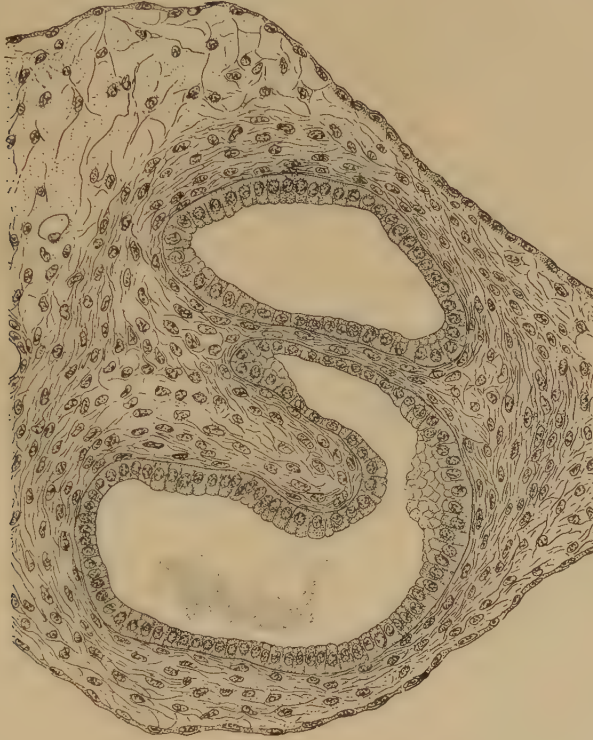


Fig. 7 Section through the posterior hepatic duct and gall-bladder of a pigeon embryo 62 mm. long. $\times 400$.

structure in an embryo 55 mm. in length. A specimen 62 mm. in length, however, showed a small persistent gall-bladder. It was of about the same diameter as the duct to which it was attached and a common musculo-fibrous tunic surrounded the

⁴ These measurements are crown-rump length taken with the neck straightened out until in line with the body.

two structures. A slender and abruptly curved cystic duct connected the gall-bladder with the posterior hepatic duct.

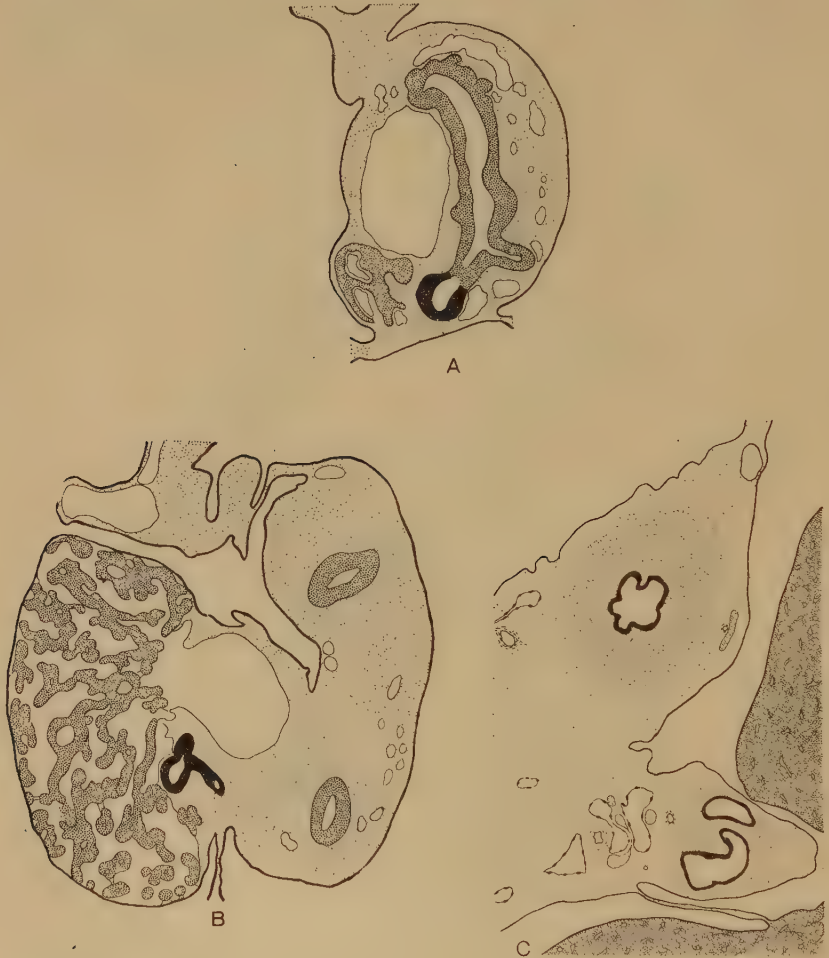


Fig. 8 Three sections showing the size of the gall-bladder in the pigeon embryo. A, embryo 6.8 mm. long (Minn. E. C. 591); B, embryo 8 mm. long (Minn. E. C. 554); C, embryo 62 mm. long. All $\times 50$.

The lining epithelium of the bladder was of a low columnar type similar to that of the duct proper, and the lumen contained a small amount of granular precipitate (fig. 7). It is evident

from this specimen that the cystic anlage may persist in some cases until about the time of hatching and perhaps later. The structure is so small that it could hardly be detected by dissection.

Attention has already been called to the great increase in the diameter of both hepatic ducts and the lack of corresponding growth of the gall-bladder. This is shown graphically in figures 8A, B and C which show longitudinal sections of the gall-bladder in embryos 6.7, 8 and 62 mm. in length respectively. These sections are all drawn at the same magnification.

RAT

Helly ('01) while studying the développement of the pancreas in the rat examined the hepatic anlagen in an extensive series of embryos ranging from 2.2 mm. to 10 mm. in length. He states that in no specimen could he find an indubitable trace of a gall-bladder. He noted that in an embryo 2.8 mm. long the primitive ductus choledochus was of considerable caliber and that the ventral portion of its wall appeared somewhat thickened. But, as this thickening passed over directly into the sprouts of the hepatic trabeculae, he did not regard it as a clear anlage of a gall-bladder. Debeyre ('04), also working on the pancreas of the rat, studied a very complete series of embryos from 11 to 21 days old. He states definitely that he found no gall-bladder in an embryo 12 days old, and makes no mention of the structure in his description of later stages.

The youngest rat embryos which I have examined were 1.75 mm. in length and 11 days old (Wistar Inst. Coll. Nos. 15346 and 15347).⁵ In these specimens the liver is already differentiated as a thickening of the ventral part of the foregut and anterior intestinal portal. The foregut is expanded a little ventrally forming a shallow hepatic pouch. From this pouch extend the earliest hepatic trabeculae which appear as solid sprouts of epithelial cells. Sagittal sections show that the liver is composed of two parts, an anterior, the true hepatic, from which the

⁵ My thanks are due to Dr. M. J. Greenman, Director of The Wistar Institute, and to Dr. C. A. Heuser, for their kindness in loaning me these preparations for a considerable period.

trabeculae are arising, and a posterior which gives rise to no trabeculae and which becomes continuous with the wall of the anterior intestinal portal. There is no evident cellular differentiation between these two parts aside from that associated with the formation of the hepatic trabeculae and the presence of a larger number of mitotic figures in the pars hepatica. A median section of the hepatic anlage at this stage is shown in figure 9.

A specimen one day older and 3.36 mm. long (Wistar Inst. Coll. No. 15363) shows the liver anlage in the form of a distinct

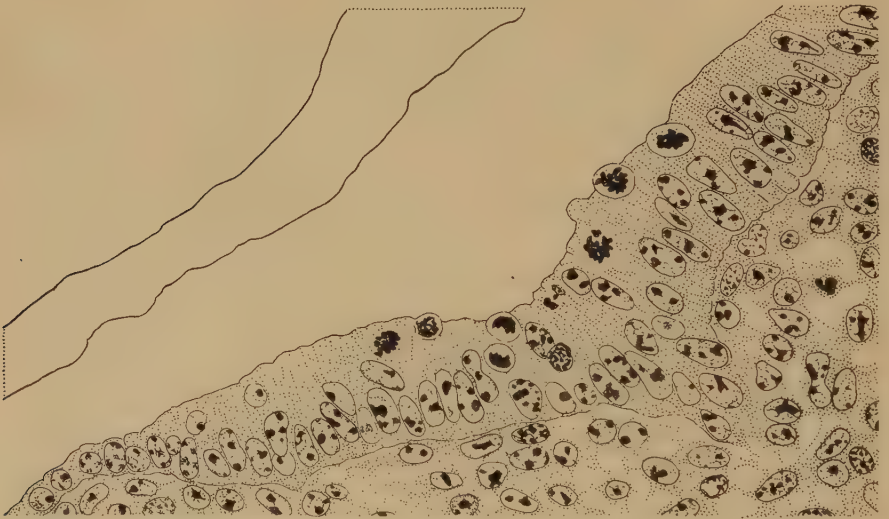


Fig. 9 Sagittal section of the hepatic pouch of an albino rat embryo 1.75 mm. long, 11 days, (Wistar Inst. Coll. No. 15346). $\times 400$.

pouch sharply marked off from the gut anteriorly and posteriorly with the forward end somewhat recessed. A large number of looping and anastomosing trabeculae arise from the anterior part of the pouch and extend some distance into the surrounding mesenchyma (fig. 10). The posterior part of the pouch gives rise to no trabeculae. In the ventral wall of this portion just in front of the posterior end of the anlagen is a slight swelling which is marked on the internal surface by a shallow pit (X, fig. 10). It extends but a little way on either side of the mid-line.

The arrangement of the cells in this swelling suggests that seen in taste-buds but the cell boundaries are not distinct. The nuclei are elongated and peripherally placed while those of the remainder of the hepatic pouch are rounded and more generally scattered. The cytoplasm is a little lighter than that of the remainder of the pouch. This structure may be the trace of a gall-bladder anlage. Its position corresponds to that of the gall-bladder in other mammals and to the thickening previously



Fig. 10 Sagittal section of the hepatic pouch of an albino rat embryo 3.36 mm. long, 12 days (Wistar Inst. Coll. No. 15363). $\times 400$. X, possible cystic anlage.

described by Helly. The arrangement of the cells is distinct but not striking. The structure seems to be present in another embryo of the same age (Wistar Inst. Coll. No. 15362) which is cut in an oblique plane but only in sagittal sections would it be possible to be sure of it. Lewis and Thyng ('08) have observed well-defined pockets resembling those of intestinal diverticula in the hepatic anlagen of pig embryos, but none were found in specimens under 7.8 mm. in length. This specimen just de-

scribed is of so much earlier a stage that I do not think the two structures can be regarded as identical. I have found no diverticula, such as are described by Lewis and Thyng in other mammals, in the bile-ducts of later rat embryos. Debeyre ('04) has described numerous pancreatic tubules arising directly from the ductus choledochus in rat embryos, but these do not appear until the fifteenth day, and their position differs from that of the swelling described above.

Older embryos examined show no trace of a gall-bladder. In embryos 5.4 mm. long the posterior part of the hepatic pouch has the form of a short wide duct bifurcated at its distal extremity. The lumen is large, particularly distally, and the cells of the ventral wall have peripherally placed nuclei but there is no cystic anlage. Other embryos (11, 13 and 18 mm. in length respectively) also show no trace of a gall-bladder. In all of these specimens the lumina of the hepatic and common bile-ducts are of unusual size.

SUMMARY AND DISCUSSION

The history of the gall-bladder is quite different in each of the three forms under discussion. In *Petromyzon* a complete biliary apparatus is formed and persists throughout the larval or *Ammocoetes* stage. At the time of the transformation of the larval to the adult form there is a total degeneration of both gall-bladder and ducts. In the pigeon the gall-bladder is developed apparently in a perfectly normal way, and later, in the majority of cases at least, is completely lost. The duct to which it is attached, persists and grows to some size. This case is complicated, however, by the presence of a larger anterior hepatic duct which opens independently into the duodenum and is never associated with the gall-bladder. In the rat there is at most but a trace of a cystic anlage in very early stages and this soon disappears. I have observed no common factors which would account for the absence of the gall-bladder in all of these examples. In the rat it is possible that the rapid and early reduction in size of the yolk-stalk which leaves the foregut wall

quite short antero-posteriorly may be a factor in inhibiting the development of the gall-bladder. In mammals generally, as compared with most lower forms, we find a distinct shortening of the liver anlage and a tendency towards consolidation of the cystic and hepatic portions. This condition is well exemplified in *Bos taurus* as shown by Pensa's ('13) recent work. The rat may be but an extreme example of this consolidation due to the conditions above mentioned.

The occasional absence of the gall-bladder in man is not explained by these observations. Barring the cases of secondary obliteration of the bile-ducts, it is possible that there are in man two types of inhibition of the development of the biliary apparatus. In one the entire pars cystica of the liver anlage is suppressed. This type would be represented by the rare cases in which both gall-bladder and bile-ducts are absent. In the second only the gall-bladder fundament is lost either early or late in development and this type would be represented by the more common anomaly of absence of the bladder only.

The large size of the bile-ducts is noticeable in both pigeon and rat embryos. This seems to be a compensation for the absence of the usual bile reservoir in the form of a definite sac. A general dilation of the bile-ducts or their local expansion into chambers capable of containing a considerable amount of bile is often but not always found in animals regularly lacking a gall-bladder. This is frequently true in cases of congenital absence of the gall-bladder in the human subject. Dilation of the bile ducts after cholecystectomy is also a common observation.

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